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## Introduction

Small, non-peptidal molecules which mimics the 3D structure of heregulin (HRG) binding domain may specifically block receptor-binding and inhibit the biological activities of the HRG and HRG receptor(s). We have used a structure-based strategy towards the discovery of small molecules as potential specific HRG antagonists. In this approach, pharmacophore (spatial arrangement of functional groups) models were constructed based upon the crucial residues in HRG that may be responsible for binding to its receptors, as well as the chemical nature and the three dimensional (3D) geometry of these residues in the 3D structure of HRG. HRG constitutes the HRG subfamily of EGF-related peptides that were originally isolated from human breast cancer cell and ras-transformed fibroblasts. HRG can stimulate proliferation and may function as an autocrine growth factor in transformed cells. Stable expression of HRG via transfection leads tumor formation in nude mice and might perform a role in progression to estrogen-independent tumor growth.

Our previous focus has been to design and develop a functional assay system to determine the mechanisms of action of the small molecules, and to discover more novel compounds with improved potency. We have achieved this goal through utilization of 32D model system in which the 32D cells have been transfected with individual or in combination of *erbB* receptors, and molecular modeling-assisted, rational design of new models based upon protein structure and these lead compounds, followed by biochemical and biological evaluations of these new candidates. Compounds that were able to block the function of HRG in binding and HRG-induced phosphorylation assays and HRG-dependent growth were considered as the **lead compounds**. Three classes of lead compounds were discovered and we have demonstrated proof of the principle that small molecules discovered through structure and computer pharmacophore search can block HRG binding and inhibit the biological activity of HRG receptors.

We have demonstrated proof of the principle that small molecules discovered through structure and computer pharmacophore model search can block HRG binding and inhibit the biological activity of HRG receptors. This type of small molecule HRG antagonists will undoubtedly have a great therapeutic potential to be used either by themselves or in combination with other conventional chemotherapeutics in the treatment of breast cancer. We believe that the combined efforts of this multi-disciplinary team will bring significantly improved small, non-peptidal molecule HRG antagonists into a phase I clinical trial in the near future.

## Specific Introduction for This Report.

Targeted disruption of a clinically relevant, oncogenic protein with small molecules is a new and attractive strategy. Our 3D-database pharmacophore search technique, and functional assay of the lead compounds is an unique and effective approach. Several small molecule compounds that inhibits the PDGF or VEGF receptor kinase activity has entered phase I or phase II clinical trials for the treatment of solid tumors. Our program has unique advantages. First, several promising lead compounds have already been discovered. Second, these lead compounds are small, non-peptidal, drug-like molecules. In fact, they are all natural products. Third, we have assembled a team with extensive experience in molecular modeling, drug design, cell and molecular biology, and pre-clinical studies in breast cancer research. This provides us

unparalleled strength for discovery of lead compounds using our effective approaches and advancement of lead compounds into pre-clinical and phase I clinical studies.

This team has extensive experience in molecular modeling, 3D-database, drug design, molecular biology and breast cancer research. We have demonstrated proof of the principle that small molecules discovered through structure and computer pharmacophore model search can block HRG binding and inhibit the biological activity of HRG receptors. It is expected that our program will generate specific, potent HRG antagonists as pre-clinical or clinical candidates in the near future. This type of small molecule HRG antagonists will undoubtedly have a great therapeutic potential to be used either by themselves or in combination with other conventional chemotherapeutics in the treatment of breast cancer. We believe that the combined efforts of this multi-disciplinary team will bring significantly improved small, non-peptidal molecule HRG antagonists into a phase I clinical trial in the near future.

If this approach is successful, it is expected that our program will generate specific, potent HRG antagonists as clinical candidates in the near future. This type of small molecule HRG antagonists will undoubtedly have a great therapeutic potential to be used either by themselves or in combination with other conventional chemotherapeutics in the treatment of breast cancer.

This project also received support in part by the Lombardi Cancer Center, and the Developmental Project of SPORE program for Breast Cancer from the Lombardi Cancer Center for the past two years.

The lead compound Rifamycin has received a patent report from US Patent and Trademark Office (PCT/US97/21474).

### Body of Report

There were three technical objectives in the original proposal. The technical objective 1 was to investigate mechanisms of action of small molecule drugs we have identified previously. We propose to utilize the 32D functional assay system to investigate: 1) whether these small molecule lead compounds specifically blocks the HRG binding to *erbB-4* homodimer and HRG-mediated biological effects via the *erbB-4* receptor; 2) whether these compounds act differently in blocking the binding of HRG to heterodimer of *erbB* receptors such as *erbB-2/3*, *erbB-1/3*, *erbB-1/4* and *erbB-2/4*; and 3) whether these compounds might interfere with other EGF-like proteins binding or signaling pathways, such as EGF, epiregulin, HRG ( or NRG-3).

The technical objective 2 was to discover additional novel lead compounds with better potency. We propose to discover additional novel lead compounds with better potency through: 1) searching the analogs of the three lead compounds; 2) designing additional pharmacophore models based on the HRG  $\exists$  EGF domain binding components and two-binding sites model of HRG; 3) pharmacophore searching of NCI database and ACD database. The advantage of having molecules that mimic two distinct binding sites is that these molecules may achieve synergistic

effect. Furthermore, we may test additive and/or synergistic effect of compounds that mimic two bindings by combinational treatment or chemically linking two molecules.

The technical objective 3 was to investigate the biological activity, specificity and therapeutic efficacy of the lead compounds. We propose to investigate lead compounds that were tested for both activity and specificity in 32D function system for their biological activities in human breast cancer cell lines. We will then test the 2-3 most promising compounds on human breast cancer xenograft on nude mice and transgenic models for therapeutic efficacy. This objective has not been accomplished as the co-PI Dr Shaomeng Wang moved to University of Michigan, Department of Internal Medicine last September. My lab is also in the middle of moving to the same department by February 2002. We plan to complete the remaining objectives and submit the final report in a year.

A search of the 3D-database of 206,000 compounds yielded 850 compounds, which were found to satisfy the pharmacophore query. The hydrophobicity of these 850 compounds was then examined and about 250 compounds were found to contain some hydrophobic moiety(ies) that could somewhat mimic those three crucial hydrophobic residues of HRG. One hundred-five compounds and were tested and three classes compounds were identified as lead compounds in the functional assays.

Most human cancer cells express more than one *erbB* receptors and ligands. Because different composition, expression and heterodimerization of various receptors might alter the binding affinity and/or internalization, it is difficult to define the exact correlation of the receptor expression and the antagonist activity of the small molecules. To overcome this difficulty, one could utilize a model cell system in which the same background cells are transfected with a particular growth factor and/or receptor as well as control genes. 32D cells from non-tumorigenic, murine hematopoietic cell line, are devoid of receptors for many growth factors (e.g., EGF, PDGF, *erbB-2/3/4*, KGF, IL-2, CSF-1, Met, Kit, etc.) and are strictly dependent on IL-3 for survival and proliferation. This IL-3 dependence, however, can be bypassed by the stimulation of signal transduction pathways initiated by the expression of specific growth factor receptors and the addition of the appropriate ligand to the culture medium. The IL-3 requirement could also be abrogated by oncogene-induced transformation such as *abl*, *src*. Compared with other commonly used mouse fibroblasts such as 3T3 cell line, there are two main advantages of the 32D model cell system. First, the 32D cells are devoid of many receptors, therefore, provide almost zero background of receptor autophosphorylation or cross-talks between receptors. Second, when 32D cells are transfected with a particular growth factor receptor, dual mitogenic and signal transduction pathways are created for the same transfectants expressing that receptor. For instance, 32D cells transfected with HRG receptor *erbB-4* will proliferate in the presence of either HRG or IL-3. With this 32D cell model system, candidate compounds can be tested for their antagonist activity and specificity in both HRG-dependent and HRG-independent growth of the same cells. Using a model cell system which does not express *erbB* receptors, we have demonstrated for the first time that cells that express either *erbB-4* or a combination of *erbB-3* with *erbB-2* exhibit enhanced response to heregulin chimerical toxin mediated cell killing. The heregulin-PE toxins have no activity on cells that express *erbB-1/EGFR* or *erbB-2* receptor. When *erbB-3* is expressed alone, heregulin-PE toxins have little or no activity. Thus, both the *erbB-4* homodimer or the *erbB-2* and *erbB-3* heterodimers are the functional receptors for ligand

heregulin. We will utilize this model system to screen small molecule antagonists that act only on the heregulin. Compounds that act as like an agonist may also be identified if cultured in the absence of HRG. Furthermore, this system will allow us to determine if some of the compounds may preferentially block the heterodimer such as *erbB-2/3*, or *erbB-1/3*, and whether some of compound may have activity against the new HRG-like ligand such as HRG (.

Single or double transfection of 32D cells by the *erbB* receptors have been established through a collaboration with Dr. Y. Yarden, which allow testing of the inhibitory potency of candidate compounds on the specific expression of *erbB* receptors. 32D cells transfected with *erbB-4* or *erbB-2/3* will proliferate in the presence of either HRG or IL-3. With this 32D cell model system, candidate compounds can be tested for their activity and specificity in various *erbB* homo or heterodimer cells. More importantly, effect of a true small molecule antagonists can be reversed by addition of excess amount of HRG.

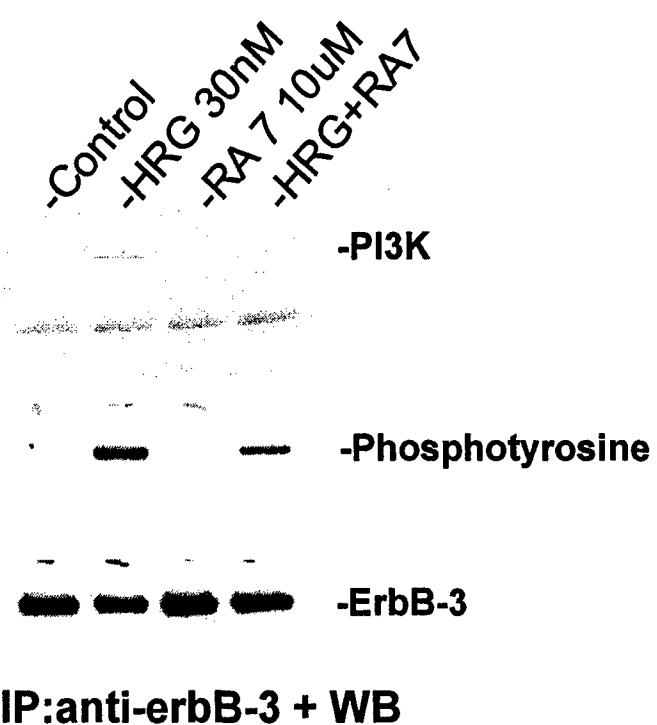
From current studies, we found that lead compound A1 inhibited only 32D/*erbB-4* cells grow in the presence of HRG, but not in the presence of IL-3. SM A1 also does not inhibit EGF stimulated growth in 32D-EGFR cells. We believe that through this assay we will be able to identify compounds that interfere only with HRG signaling pathway.

-Control  
-HRG 30nM  
-RA 1 10uM  
-HRG+RA1

— — -PI3K

-ErbB-3

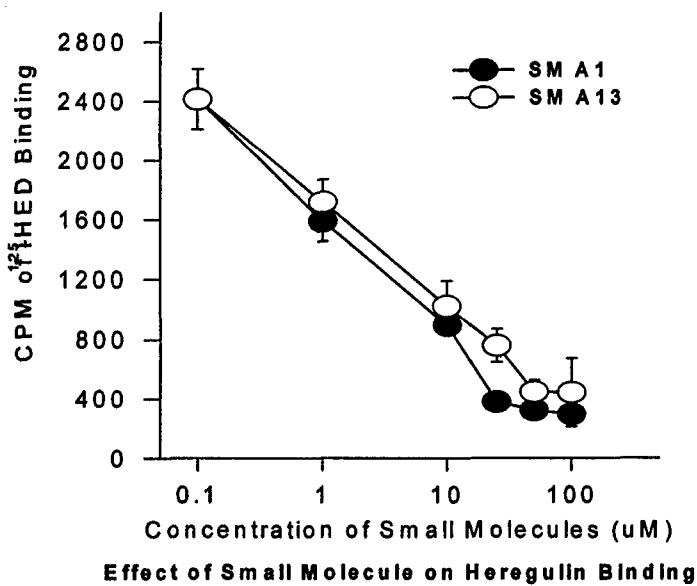
**IP:anti-pTY + WB**

**IP:anti-erbB-3 + WB**

In the biochemical studies, we have used the cells that express little phosphorylated receptors and stimulated phosphorylation of receptor by addition of HRG into the culture medium. However, at the presence of the HRG inhibitor RA7, the activation of the receptor is totally blocked. We further demonstrated such inhibition is an reversible event since the addition of extra amount of HRG could partially reverse the small molecule's effect. The total protein expression of the receptor was not affected by HRG or small molecule inhibitors.

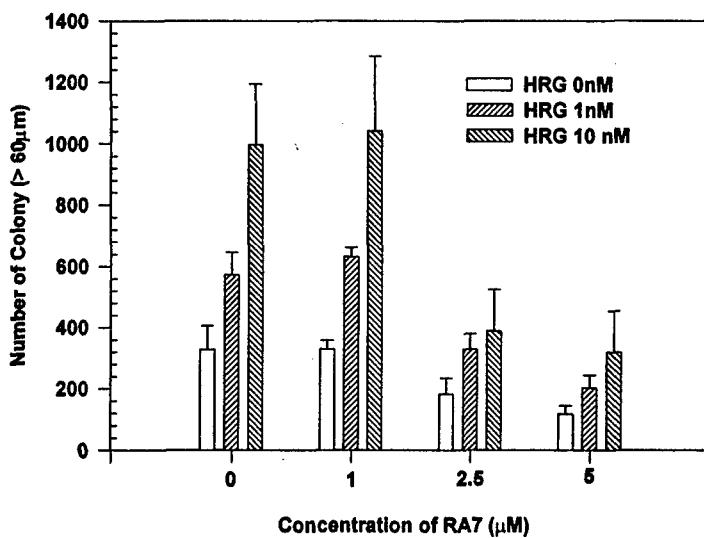
**More than 10-fold improvement on inhibition of receptor binding.**

Screening of 30 more rifamycin analogs in dose-dependent receptor-binding assays were performed and four analogs were found to have  $IC_{50}$  about 1-10  $\mu$ M. This represent more than 10 fold improvement for initial rifamycin compounds in the receptor binding assay, which was between 100-200  $\mu$ M.



**Inhibition of HRG-induced soft-agar colony formation and reverse with addition of excessive amount of HRG.**

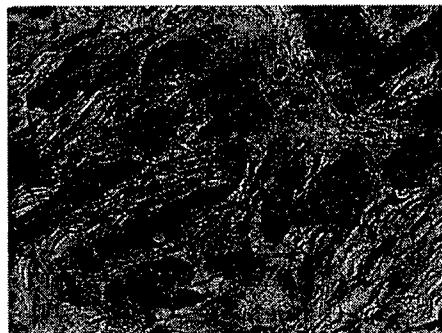
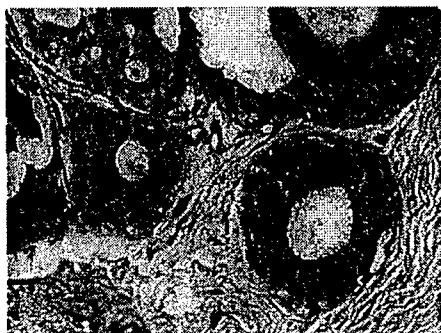
We have made recombinant HRG- $\beta$ 1 which induce phosphorylation in a dose-dependent manner over range of 0.01 nM to 1 uM in MCF-7 or T47D cells. The recombinant HRG- $\beta$ 1 can induce soft agar colony formation of MCF-7 in a dose-dependent manner. The ability of the small molecules to block HRG-induced soft-agar colony formation was tested by co-incubation



with 1nM, 10nM HRG and 1uM, 2.5uM and 5uM of lead compound SM-A1(RA7). An example of dose dependent inhibition of HRG-induced colony formation is shown. The specificity of HRG antagonist activity was evidenced by reverse of inhibition with excessive amount of HRG.

#### **Elevated Expression of HRG in Human Breast Cancer Tissues.**

A specific mAb that recognize HRG  $\beta$  in the paraffin tumor samples was found and used to carry out immunohistology staining of total 64 human breast cancer samples. Thirty one tumor samples (49%) were found to stain positively for HRG  $\beta$  in tumor cells, including the DCIS and invasive ductal carcinomas. Two examples of such staining of HRG  $\beta$  in human breast cancer cells were shown below.



#### **Significance.**

HRG constitutes the HRG subfamily of EGF-related peptides that were originally isolated from human breast cancer cell line MDA-MB-231, and ras-transformed Rat-1 fibroblasts. HRG can stimulate proliferation and may function as an autocrine growth factor in transformed mammary epithelial cells. Stable expression of ligand HRG via transfection leads tumor formation in nude mice and might perform a role in progression to estrogen-independent tumor growth. Furthermore, HRG induces *in vivo* lobuloalveolar development of mammary gland, and in MMTV-HRG transgenic mice, HRG induces persistence of terminal end buds and mammary adenocarcinoma, and 50% developed Harderian hyperplasia (a benign tumor). Clinically, elevated expression of HRG play a role in breast cancer growth and progression via autocrine loop and is associated with less favorable disease outcome. Thus, the discovery of these lead compounds represents an exciting and important step in the development of the small molecule, specific HRG antagonists as potential clinical candidates in the prevention and treatment of breast cancer.

Immunostaining studies show that *erbB-3* is overexpressed in breast cancer, and others. The expression of *erbB-4* is elevated in breast cancer cell lines, and we have found its overexpression in invasive ductal carcinoma and DCIS of breast but not in the nearby normal breast cells. More recently, three more heregulin-like genes, NRG2, NRG 3 and HRG (, interact with *erbB-3/4* or *erbB-4* only, have been reported. Our laboratory have been engaged in projects aimed at development of *erbB* receptor-mediated therapy in breast cancer. Expression of the EGFR, *erbB-2*, *erbB-3*, *erbB-4*, HRG and *in vivo* tumorigenicity of breast cancer cells and their response to the heregulin-PE toxins mediated killing have been well characterized.

Recently, it has been shown that it is indeed possible to discover and identify small molecules that can disrupt the protein-protein interactions. One example of such is published

recently by Li *et al.* in the discovery of the small molecule that effectively block the stable association between CD4 and MHC II protein. These small molecules appears to specifically target the CD4/MHC II complex and showed inhibition of immune responses in animal models and allograft transplant rejection. Therefore, these CD4 inhibitors may be developed as novel immunotherapeutics. We believe that the design and discovery of growth factor receptor antagonists will become a very active and fruitful research area in the near future.

### Future Plan.

It is anticipated that we might discover and furthermore, synthesize lead compounds with potency and toxicity meet criteria for clinical trial studies in near future. We would then need to have the scale-up production with GMP standard to proceed with IND filing. Lombardi Cancer Center Developmental Therapeutic Program has run many clinical trials for novel anti-cancer drugs and will assist us in the design and execution of clinical trial study.

### KEY RESEARCH ACCOMPLISHMENTS:

Bulleted list of key research accomplishments emanating from this research.

- ◆ Characterized the biological activity and specificity of small molecule antagonist of growth factor Heregulin in 32D model system;
- ◆ Biochemical and biological studies of the small molecule antagonist of HRG;
- ◆ Provided the proof-of-concept for the small molecule inhibitor studies of HRG interruption and also the success of funding from the Developmental Project of the SPORE Program from the Lomardi Cancer Center.
- ◆ Discovered more potent small molecule antagonist for HRG with further cell based inhibition activities.
- ◆ Report of one allowed patent application of small molecule antagonist of HRG

### REPORTABLE OUTCOMES:

Provide a list of reportable outcomes to include:

- manuscripts, abstracts, presentations;

Gao Y, Voigt J, Wu JX, **Yang D\***, Burke TR Jr. Macrocyclization in the design of a conformationally constrained Grb2 SH2 domain inhibitor. *Bioorg Med Chem Lett* 2001 Jul 23;11(14):1889-92

Burke TR Jr, Yao Z, Gao Y, Wu JX, Zhu X, Luo JH, Guo R, **Yang D\***. N-Terminal carboxyl and tetrazole-containing amides as adjuvants to Grb2 SH2 domain ligand binding. *Bioorg Med Chem* 2001 Jun;9(6):1439-45

Gao, Y., Luo, J., Yao, Z., Voigt, J., Guo, R., Zuo, H., **Yang, D\***. and Burke, T.R. Inhibition of Grb2 SH2 domain binding by non-phosphate containing ligands 2,4-(2-malonyl) phenylalanine as a potent phosphotyrosyl mimetic. *Journal of Medicinal Chemistry*, 43(5):911-920, 2000. (shared senior author, and my postdoc is shared first author).

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Mandler, R., Wu, C., Ho, D., Sausville, E.A., Roettinger, A.J., Newman, D.J., King, C.R., **Yang, D.**, Lippman, M.E., Vitetta, E.S., Landolfi, N., Brechbiel, M.W., Waldmann, T.A. Conjugation of geldanamycin to an anti-HER2/neu monoclonal antibody augments the tumor-targeted inhibitory effects of the antibody on human breast carcinoma cells. **Journal of Nat. Cancer Inst.**, 92:1573-81, 2000.

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Gao, Y., Yao, Z., Voigt, J., Luo, J., **Yang, D\***. and Burke, T.R. Novel phosphotyrosyl mimetics for the preparation of potent small molecule Grb2 SH2 domain inhibitors. "Peptides for the New Millennium: proceedings of the 16<sup>th</sup> American Peptide Symposium", G. B. Fields, J.P. Tam, and G. Barany (Eds.), Kluwer Academic Publishers, Dordrecht, The Netherlands, 10-13, 1999.

Long, Y.Q., Lung, F.D., Voigt, J., Yao, Z., Burke, T.R., **Yang, D\***., Luo, J., Guo, R., King, C.R., and Roller, P.P. High Affinity nonphosphorylated cyclic peptide inhibitors of Grb2-SH2/growth factor receptor interaction. "Peptides for the New Millennium: proceedings of the 16<sup>th</sup> American Peptide Symposium", G. B. Fields, J.P. Tam, and G. Barany (Eds.), Kluwer Academic Publishers, Dordrecht, The Netherlands, 102-105, 1999.

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#### RELATED ORAL PRESENTATIONS:

89th Annual Meeting of American Association for Cancer Research, New Orleans, USA, "Discovery of small molecule antagonists of heregulin/heregulin receptors through the computer-based pharmacophore 3-D database search", Pharmacology and Experimental Therapeutics, Novel Agents and Targets Minisymposium, March 30, 1998

#### RECENT RELATED ABSTRACTS

**Dajun Yang**, Yan Ling, Istvan Enyedy, Jingson Wang, Xiaofeng Zhu, Zhujun Yao, and Shaomeng Wang. Selective Inhibition of ErbB-2 (Her-2/neu) Kinase and Anti-tumor Activity with a Novel Class of ErbB-2 Specific Kinase Inhibitor. **Late-Breaking Abstract**. 11<sup>th</sup> NCI-EORTC-AACR Symposium on New Drugs in Cancer Therapy, Amsterdam, November, 2000.

Ribo Guo, Yan Ling, Juliet Luo, Zhu-Jun Yao, Hong Zuo, Yang Gao, James Kelley, Johannes H. Voigt, C. Richter King, Terrence R. Burke, Jr. and **Dajun Yang**. Inhibiting Grb2 SH2 Domain Interactions is Cytostatic and Enhances sensitivity to Chemotherapeutic Drugs in Human Breast Cancer Cells Overexpressing Her-2/neu. **Proc. Amer. Assoc. Cancer Res.**, Vol. 41, #3068, pp481, 2000. (Poster Discussion)

Burke, T.R., Gao, Y., Yao, Z., Voigt, J., Luo, J., and **Yang, D.** Potent Non Phosphate-Containing Grb2 SH2 Domain Inhibitors. "Peptide Chemistry 1999: proceedings of the 36<sup>th</sup> Japanese Peptide Symposium", pp 100-104, 1999.

Wang, S., Guo, R., Zuo, H., Wang, J.S., Lippman, M.E., and **Yang, D.** ErbB-3 and ErbB-4 Receptors Ligand Heregulin Selective Small Molecule Antagonists for Breast Cancer Therapy 7<sup>th</sup> SPORE Investigators' Workshop, NCI, Rockville, July 11-13, 1999. P12.

Wang, S., Guo, R., Tan, J., Payne, J., Milne, G.W.A., Lippman, M.E., and **Yang, D.** Discovery of Small Molecule Antagonists of Heregulin/Heregulin Receptors Through the Computer-based Pharmacophore 3-D Database Search. **Proc. Amer. Assoc. Cancer Res.**, Volume 39, #1209, pp77, 1998.

S. Wang, J.Tan, J.Payne, G.W.A. Milne, M.E.Lippman and **D.Yang\*** Discovery of Small Molecule Antagonists of Heregulin/Heregulin Receptors Through the Computer-based Pharmacophore 3-D Database Search. 5th Annual SPORE Meeting, July 1997.

S. Wang, J. Tan, J. Payne, G.W.A. Milne, M. E. Lippman and **D. Yang\*** Discovery of Small Molecule Antagonists of Heregulin/Heregulin Receptors Through the Computer-based Pharmacophore 3-D Database Search. 4th Annual International Conference on New Advances in "Peptidomimetics and Small Molecule design", March, 1997.

\*Those are related publications in the same target use small molecule approaches or biological studies of heregulin.

- patents and licenses applied for and/or issued;  
1. PCT/US97/21474, Title: Heregulin antagonists and methods for their use  
Inventors: **Yang, D.**, Wang, S., Lippman, M.E., and Kozikoski, A.
  
- degrees obtained that are supported by this award;  
Not applicable.
- development of cell lines, tissue or serum repositories;  
Not applicable.
- informatics such as databases and animal models, etc;  
Not applicable.
- funding applied for based on work supported by this award;  
1. SPORE (Specialized Program of Research Excellence) Breast Cancer, NIH 1P50CA5818  
**Role: Developmental Project Leader**  
Title: Small molecule antagonist of heregulin and heregulin receptors for breast cancer  
Funding Agent: National Cancer Institute/NIH, 1998-2000, annual direct \$50,000  
This is a developmental project focus on the testing the novel small molecule antagonists of heregulin as anti-tumor and combination therapy in breast cancer.
  
- 2. Innovative Research and Development Award (IDEA) grant  
**Co-Principal Investigator** (with Dr. Shaomeng Wang)  
Title: Structure based design of erbB-2 selective small molecule kinase inhibitors  
US Army Medical Research and Materiel Command, BC990340  
2000-2003, annual direct \$75,000, annual indirect \$ 40,000  
This grant is to discover and design the small molecule inhibitors that selectively inhibit the erbB-2 kinase activity.
  
- employment or research opportunities applied for and/or received on experiences/training supported by this award.  
Not Applicable.

**CONCLUSIONS:** Summarize the results to include the importance and/or implications of the completed research and when necessary, recommend changes on future work to better address

the problem. A "so what section" which evaluates the knowledge as a scientific or medical product shall also be included in the conclusion of the annual and final reports.

The EGF receptors (or *erbB*) are prototypes for a family of structurally related transmembrane proteins that play a role in pathogenesis of cancer. Members of this family including the EGFR, *erbB-2/neu*, *erbB-3* and *erbB-4* gene, are overexpressed in at least 60-70% of breast cancers. HRG has been found to stimulate proliferation of mammary epithelial cells both *in vitro* and *in vivo* and elevated expression in human breast cancers is associated with tumor progression and poor prognosis.

We have developed a novel, structure-based strategy towards the discovery of small, non-peptidal molecules as potential specific HRG antagonists. In this approach, pharmacophore (spatial arrangement of functional groups) models were constructed based upon the crucial residues in HRG that may be responsible for binding to its receptors, as well as the chemical nature and the three dimensional (3D) geometry of these residues in the 3D structure of HRG. An effective, computerized 3D-database pharmacophore search technique was then employed to identify small molecules in the NCI 3D-database that resemble these pharmacophores. In essence, this search detected compounds whose 3D structures mimic the binding domain of the HRG. Thus far, three classes of **lead compounds** have been discovered as specific HRG antagonists in receptor binding competition, HRG-induced phosphorylation assays. Addition of excessive amount of HRG can reverse the inhibitory effects mediated by the small molecules. These are the results of testing 105 candidates, selected from the 3D-database pharmacophore search results of 206,000 compounds in the NCI 3D-database. To date, there is no report that small molecules can act as specific antagonists of HRG.

Targeted disruption of a clinically relevant, oncogenic protein with small molecules is a new and attractive strategy. Our 3D-database pharmacophore search technique, and functional assay of the lead compounds is an unique and effective approach. Several small molecule compounds that inhibits the PDGF or VEGF receptor kinase activity has entered phase I or phase II clinical trials for the treatment of solid tumors. Our program has unique advantages. First, several promising lead compounds have already been discovered. Second, these lead compounds are small, non-peptidal, drug-like molecules. In fact, they are all natural products. Third, we have assembled a team with extensive experience in molecular modeling, drug design, cell and molecular biology, and pre-clinical studies in breast cancer research. This provides us unparalleled strength for discovery of lead compounds using our effective approaches and advancement of lead compounds into pre-clinical and phase I clinical studies.

**REFERENCES:** List all references pertinent to the report using a standard journal format such as *Science*, *Military Medicine*, etc.

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**APPENDICES:** Attach all appendices that contain information that supplements, clarifies or supports the text. Examples of appendices include journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.

We will mail these appendices separately if needed.

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